



## Root growth effects on soluble C and P in manured and non-manured soils

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### Abstract

There is limited research on relationships between root characteristics and soil chemical properties and processes. Because previous studies have shown specific C compounds may release previously sorbed P and make P more plant-available, crops which contribute to high soil C levels could play an important role in soil P cycling. The objectives of this study were to determine (1) whether rotation crops had different amounts of root growth, (2) whether different amounts of root growth among the crop species could be related to different levels of soluble soil C and (3) whether there were differences in P concentration among the soils under different crops that could be related to soluble C soil concentration. Roots and soil from potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.), soybean (*Glycine max* (L.) Merr.), and a forage consisting of alfalfa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.) were sampled from the Aroostook Research Farm in Presque Isle, Maine, during the summers of 2003 and 2004 to determine root length density (RLD) and soluble C and P concentrations. Half of the sampled plots were amended with beef manure and half were not amended. Barley and forage consistently had higher RLD than potato or soybean crops. Barley and forage typically had higher concentrations of soluble soil C than potato or soybean, but the differences were significant at only three of the five sampling dates. RLD was significantly correlated to soluble C ( $r=0.56$ ) only for amended soils on the August 2003 sampling date. For other dates  $r$  values were non-significant and ranged from 0.32 to 0.49. As with soil C, soluble soil P levels were typically higher in barley and forage than in potato or soybean crops. Significant differences were detected at four of the five sampling dates. Correlations between soluble C and soluble P were significant at two of the five sampling dates ( $r=0.58$  and  $0.62$ ) in amended soils and one of five sampling dates ( $r=0.80$ ) in unamended soils. Although the correlations between RLD and soluble C were not significant at every sampling date, the August 2003 data do suggest a possible effect of roots on soluble C. In addition, significant correlations between soluble C and soluble P at several sampling dates suggest a relationship between these parameters. Therefore cropping systems that include crops with higher amounts of root growth may promote increased soluble soil C levels and enhance P bioavailability.

**Abbreviations:** ICP-AES – inductively-coupled plasma atomic emission spectroscopy; DPS – degree of phosphorus saturation; DI – deionized;  $P_i$  – inorganic P;  $P_o$  – organic P;  $P_{tot}$  – total P; RLD – root length density

### Introduction

Addition of organic residuals at relatively high rates may be necessary to maintain or improve

soil organic matter levels in intensively cropped systems. Manure, a residual which offers many potential soil benefits, is available to some growers. In addition to carbon (C), manure contains significant amounts of plant-available nitrogen (N) and phosphorus (P), which can be recycled

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in land application. Manure application rates are based on crop N needs and estimated rates of manure N supply. Animal manures have an approximate N:P ratio of 4, while many crops have an N:P ratio of 8 (Sharpley et al., 1994). Therefore, basing manure application on N supply typically results in P additions in excess of crop needs. The excess P builds up in soils, increasing their potential to contribute soluble and particulate P to surface waters with the occurrence of erosion or overland flow and, thus, enhance P-driven eutrophication. The need to dispose of animal manure, and its potential to improve soil quality and increase crop yields, are in conflict with the likely increases in soil P due to manure application and its link to water quality deterioration.

There are many suggestions in the literature that the application of organic material to soil can increase P availability to crops and, thus, the efficiency of crop use of residual soil P. Small organic acids, either added with the organic material or released during decomposition, are thought to be partially responsible for increases in P availability following organic matter additions. A wide range of materials including acetic, citric, formic, fumaric, lactic, maleic, malonic, oxalic, succinic, and tartaric acids, have been found in soils, litter, compost, and manure (Bolan et al., 1994; Baziramakenga et al., 1995). Three mechanisms have been proposed to explain the influence of soluble organic matter and organic acids on P sorption (Iyamuremye and Dick, 1996). First, the organic molecules may be sorbed by soil minerals, competing with P for sorption sites. Second, the soluble organic matter may complex with surface-bound Al or Fe to form soluble organic-metal compounds, releasing previously sorbed P. The activity of the metal in solution would then be reduced, which would favor the dissolution of other metal compounds, including those containing P. Finally, organic matter may be sorbed to the surface of soil particles at sites other than those that sorb P. This could increase the surface negative charge of the particle, making P species less attracted to the soil and more likely to remain in solution. Ohno and Crannell (1996) observed that low concentrations of hairy vetch (*Vicia villosa* L.) and crimson clover (*Trifolium incarnatum* L.) extracts

inhibited P sorption which they attributed to competitive adsorption of C compounds. In a study of organic residuals (dairy manure and cheese whey) added to eroded calcareous soils and subsoils, Robbins et al. (2000) found that soluble P levels were generally more strongly correlated with soil organic C levels than soil test P levels. They suggested that organic matter from manure coats adsorption sites and decreases P sorption.

In addition to increasing soluble organic matter in soils, manure additions induce other soil chemical changes which may influence P solubility. In particular, applications of manure can reduce the sorption of subsequent applications of P by loading sorption sites with manure-derived P (Iyamuremye et al., 1996). In a previous study (Erich et al., 2002) we hypothesized that manure and compost additions would increase P solubility and decrease P sorption due to higher levels of soluble organic ligands in manure and compost amended plots. However, because the amendments added large amounts of labile P as well as soluble C, no direct evidence of such an effect was detectable.

A rotational cropping system study offered the opportunity to examine the effects of soluble soil C on P solubility among soils with a recent history of receiving similar amounts of either inorganic fertilizer P or labile P from manure applications. We sampled plots planted with four different crops, both amended with manure and non-amended. Our first objective was to determine whether crops altered soluble organic C concentration in soil due to different amounts of root growth among the crop species. We hypothesized that different crops would have different amounts of root growth and also that there would be a positive correlation between root growth and soluble soil C concentration. Our second objective was to determine whether there were differences in P concentration among the soils under different crops and whether observed differences were related to soluble C concentration. We hypothesized that crops with higher levels of soluble C in the soil around their roots would also have higher levels of soluble P, i.e. there would be a positive correlation between soluble C and soluble P in soil.

## Materials and methods

### *Experimental design*

The experimental plots used are a subset of the Maine Potato Ecosystem Project, which was initiated at the Aroostook Research Farm in Presque Isle, Maine in 1991 (Gallandt et al., 1998). In total, the research plots cover 5.8 ha, with the dominant soil type being a gravelly, well-drained Caribou loam (fine-loamy, mixed, frigid, Typic Haplorthod). The crops studied were grown in a 4-year rotation cycle (potato (*Solanum tuberosum* L., Atlantic variety) – soybean (*Glycine max* (L.) Merr.) – barley (*Hordeum vulgare* L.) underseeded with alfalfa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.) – forage, consisting of an alfalfa/timothy mix) in a randomized complete block design with two factors (crop type and amendment) and four replications. All four crops were sampled in 2003. In 2004, potato, barley, and forage crops were sampled; soybean was not sampled due to poor early-season growth. All plots are 14.6×41 m.

There were 32 plots sampled, out of which 16 were designated 'amended' (with beef manure) and 16 'not amended'. Potato plots were amended with 67 Mg ha<sup>-1</sup> of manure, barley plots with 45 Mg ha<sup>-1</sup>, and forage plots with 27 Mg ha<sup>-1</sup> after the first cutting, while no manure was applied to the amended soybean plots. Amended plots received no synthetic fertilizer except for 78 kg N ha<sup>-1</sup> applied to potato at planting. At-planting fertilizer (kg ha<sup>-1</sup> N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O) in the unamended plots was 134-134-134 (potato), 78-0-0 (barley), and 34-34-34 (soybean). Plots were tilled to approximately 25 cm depth in the fall by chisel plowing (except the forage plots to be overwintered). In the spring prior to planting they were disked twice to approximately 15–20 cm depth depending on conditions and then harrowed once to approximately 15 cm depth (except the forage plots which had overwintered). Potatoes were cultivated and hilled in July.

### *Soil sampling and analysis*

Soil samples were taken before planting on 19 May 2003 and again on 11 May 2004. Fifteen 1.5-cm diameter cores were taken to a depth of approximately 30 cm from each plot. All fifteen

samples were combined and mixed in a plastic bag to create a single sample per plot and transported back to the lab.

The field-moist samples were sieved (4 mm) and soil water content was determined gravimetrically for each sample. Field-moist samples (15 g dry weight equivalent) were added to deionized (DI) water in plastic 50-mL centrifuge tubes. The total amount of water (soil water plus added water) in the tubes equaled 30 g. They were then shaken at 95 rpm for 1 h and centrifuged for 30 min at 1610×g. After centrifugation, pH was determined using an Orion 8103 Ross electrode. Following pH determination, the samples were filtered through 0.4 µm Whatman polycarbonate filters. Aliquots of the filtrate were analyzed for total dissolved C by combustion using a Shimadzu (Braintree, MA, USA) TC-5000 analyzer, and separate aliquots were analyzed for total P (P<sub>tot</sub>) by inductively-coupled plasma atomic emission spectroscopy (ICP-AES) using a Thermo Jarrell Ash (Franklin, MA, USA) IRIS 1000.

### *Root sampling and analysis*

Roots were sampled on 11 and 22 August 2003 in unamended and amended plots, respectively. In 2004, amended and unamended plots were sampled together on 20 and 21 July and again on 24 and 25 August. Twelve large diameter (8 cm) cores were taken in all plots to a depth of approximately 30 cm. Simple random sampling schemes were applied for both the barley and forage plots. However, since both potatoes and soybeans are row crops, stratified random sampling procedures were used. In potato plots, a sampling scheme similar to that outlined by van Noordwijk et al. (1985) was used. Three plants were selected randomly within the plots and four cores were taken surrounding each plant. In the soybean plots, six plants were randomly selected, and two cores were taken surrounding each plant. For both potato and soybean, one core was taken essentially on top of the plant; the other cores were taken 7–10 cm from the center of the plant. All samples were stored in coolers and transported back to the laboratory for analysis.

Samples were stored at 10 °C for less than 24 h prior to homogenization. Homogenization and subsampling procedures were similar to Schroth and Kolbe (1994). All 12 cores from each

plot were spread out on a plastic tarp in a cool building where they were combined and mixed thoroughly. Coarse roots (approximately greater than 2 mm diameter) were removed and retained in the subsample since coarse roots are not adequately represented in a small subsample. A preliminary experiment determined that a subsample equal to 5% of the total soil weight was sufficient to get a representative estimate of root length for all crops (data not shown). In addition to the 5% subsample taken for root measurements, an additional subsample was taken for soil chemical analysis, as described previously. The subsamples taken for root measurements were washed using a hydropneumatic elutriation system as described by Smucker et al. (1982). Samples were run through the elutriator for eight min using a 410  $\mu\text{m}$  primary sieve. Approximately 150  $\text{cm}^3$  of soil was put in one washing chamber at a time. After washing, the cleaned roots were rinsed from the collection sieve into plastic bags containing a small amount of 95% ethanol and stored at approximately 4 °C for no longer than 48 h prior to hand-cleaning.

After initial washing, the roots were subjected to a hand-washing procedure to remove as much organic debris as possible. Roots were put on a 595  $\mu\text{m}$  sieve and the sieve was dunked in a tray of DI water several times to remove as much remaining soil mineral matter as possible. The roots were then rinsed into a large beaker of DI water and large floating straw, grass, and other organic debris was decanted or hand-picked out of the beaker. In 2004, the roots were further cleaned by spreading the roots and debris in a clear plastic tray (35×45 cm) and hand-picking out any remaining organic debris and soil mineral matter. After cleaning, roots were stored in the refrigerator submerged in a mixture of water and ethanol.

For root length measurements, the line-intercept method described by Newman (1966) and modified by Tennant (1975) was used in 2003. The number of vertical and horizontal intersections was multiplied by a conversion factor of 0.7857 to estimate the total root length (in cm). In 2004, root length was measured using a WinRHIZO scanner (Regeant Instruments, Quebec, Canada). Prior to scanning, roots were stained with new methylene blue N in order to enhance visibility on the scanner (Harris and Campbell,

1989). After 24 h of staining, the roots were rinsed, submerged in DI water, and stored at 4 °C until scanning. Preliminary tests on the WinRHIZO scanner revealed that a total root length per unit area greater than 2.66  $\text{cm cm}^{-2}$  resulted in greater than 5% error in root length measurements. All scans were completed using the largest possible tray size (20×30 cm) with the total root length on the tray around 1200 cm (2  $\text{cm cm}^{-2}$ ). Each tray was scanned three times and an average of the three scans was computed. A filter was applied to each scan, excluding objects with a length/width ratio smaller than five.

#### *Soil and manure chemical characterization*

Soil samples were collected from each plot during the fall of 2002 and 2003 for soil test characterization. Soils were air-dried, sieved (< 2 mm), and characterized using the standard methods of the University of Maine Soil Testing Service (Hoskins, 1997) using modified Morgan extractant (McIntosh, 1969). Five gram of soil and 20 mL of buffered (pH 4.8) 1.25  $\text{mol L}^{-1}$  ammonium acetate was shaken for 15 min and then filtered through Whatman #42 paper filters. The filtrate was analyzed by ICP-AES. Soil pH was determined using a 1:1 DI water:soil ratio, and organic matter was estimated by loss on ignition at 375 °C for 2 h. Soil P fractions were determined using soil collected in 2003. Water soluble P was determined as previously described. Phosphorus saturation was determined using the following equation:

$$\text{DPS}(\text{degree of phosphorus saturation}) \\ = 100 * [\text{P}_{\text{ox}} / 0.5(\text{Al}_{\text{ox}} + \text{Fe}_{\text{ox}})]$$

where  $\text{Al}_{\text{ox}}$  and  $\text{Fe}_{\text{ox}}$  are the amounts of non-crystalline Fe and Al estimated by ammonium oxalate dissolution, and  $\text{P}_{\text{ox}}$  is the amount of P solubilized by ammonium oxalate. Soils (0.4g) were reacted with 40 mL ammonium oxalate (0.2 M, pH 3) in the dark for 4 h. The tubes were then centrifuged for 30 min at 1610×g. The supernatant was filtered through 0.45  $\mu\text{m}$  Whatman polycarbonate filters and Al, Fe, and P concentrations determined by ICP-AES.

Resin-extractable P was determined by the method of Guertal et al. (1991). Briefly, 2 g of soil was combined with 2 g washed and air-dried

resin (Dowex AG1-X8, 20–50 mesh) and 20 mL DI water in a 50 mL centrifuge tube and shaken for 16 h on a wrist-action shaker. After shaking, the resin was separated from the soil using density-gradient centrifugation described by Thien and Myers (1991). Phosphorus was desorbed from the resin using 25 mL of 10% NaCl solution (Olsen and Sommers, 1982). Extracts were analyzed for inorganic P ( $P_i$ ) by ion chromatography using a Dionex (Sunnyvale, CA, USA) ICS-1000 chromatograph. Soil organic P ( $P_o$ ) was determined by the ignition method (Kuo, 1996), using modifications described by Erich et al. (2002).  $P_{tot}$  was determined using microwave digestion and analysis by ICP-AES. Manure P fractions were determined using a procedure similar to Dou et al. (2000). Briefly, dried, ground manure (0.3 g) was added to 30 mL DI water in a 50-mL centrifuge tube (3 replicates), shaken for 1 h, centrifuged at 1230×g, filtered through a 0.45  $\mu$ m Whatman PC Nucleopore filter, and analyzed by ICP-AES. The manure residue was then sequentially extracted with 0.5 M  $NaHCO_3$ , 0.1 M NaOH, and 1.0 M HCl and analyzed as described above. Phosphorus not extracted by any of the extractants was assumed to be residual P.

### Statistical analysis

Data were analyzed using an analysis of variance (ANOVA) at an alpha level of 0.05 in a randomized complete block design. Statistical significance was determined using the general linear model (GLM) procedure in the SAS statistical program. Residuals were examined for normality (Shapiro-Wilks test) and equality of variances (Levene's test). Variables not meeting the ANOVA assumptions were transformed using the appropriate transformation. At all dates, mean

separation was performed using a Fisher's protected Least Significant Difference (LSD) test. Each date was analyzed separately, as time was not a factor in this study.

All correlation coefficients were determined using Basic Pearson correlations in SAS at an alpha level of 0.05. Individual plot means for each variable were used to determine correlation coefficients. In August 2003, thirty-two observations were used in the correlation analysis. Twenty-four observations were used in July and August 2004.

## Results and discussion

### Manure and soil characterization

The nutrient composition of the beef manure applied to amended plots in 2003 and 2004 is shown in Table 1 and Figure 1. Average manure pH was 9.0 in 2003 and 8.0 in 2004 (Table 1). The high pH of the manure samples is consistent with other values reported in the literature (Eghball, 2002; Eghball et al., 1996; He et al., 2004), and is primarily due to carbonates and bicarbonates in livestock diets (Barnett, 1994; Whalen et al., 2000). Water extractable P and total labile P (water plus bicarbonate extractable) concentrations were approximately twice as high in 2003 as in 2004 (Figure 1). The lower percentage of labile P in 2004 is probably due to the higher levels of Ca found in the 2004 manure sample (Table 1). Calcium phosphates decrease in solubility with increasing pH (Lindsay, 1979). Levels of residual P were similar in 2003 and 2004; however levels of HCl- and NaOH-extractable P were higher in 2004 than in 2003. Residual P is the least likely to be solubilized and mobilized in soil and HCl- and NaOH-extractable forms of P are thought to be intermediate in lability. The

Table 1. Nutrient composition of beef manure applied to experimental plots during 2003 and 2004

Year	TN <sup>a</sup> (g kg <sup>-1</sup> DM)	NH <sub>4</sub> -N (g kg <sup>-1</sup> DM)	P (g kg <sup>-1</sup> DM)	K (g kg <sup>-1</sup> DM)	TC <sup>b</sup> (g kg <sup>-1</sup> DM)	Ca (g kg <sup>-1</sup> DM)	% H <sub>2</sub> O	pH
2003	18.7 (8.0) <sup>c</sup>	2 (1.1)	5.1 (1.7)	15.6 (8.1)	332 (88.6)	5.1 (2.4)	76 (5.7)	9 (0.2)
2004	10.3 (2.8)	0.3 (0.3)	2.4 (0.2)	4.9 (1.2)	174 (52.7)	33.6 (3.5)	59 (11.8)	8 (0.4)

<sup>a</sup>TN = Total Nitrogen.

<sup>b</sup>TC = Total Carbon.

<sup>c</sup>Standard deviation in parentheses,  $n = 3$ .

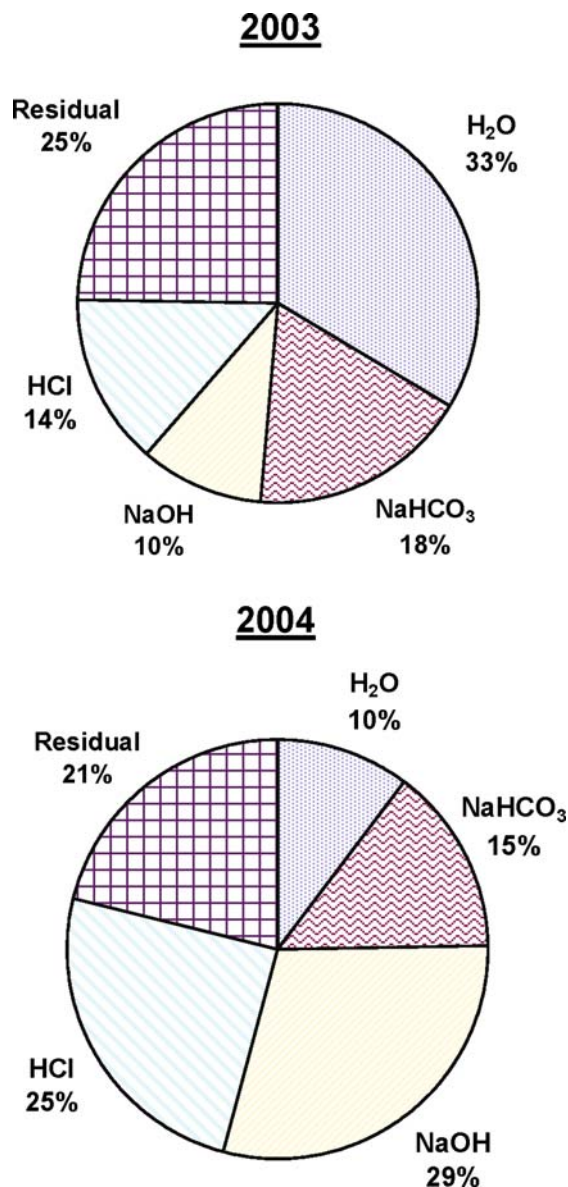


Figure 1. Phosphorus fractions in manure applied to Maine potato ecosystem amended plots in 2003 and 2004. Labile P is equal to water extractable and bicarbonate extractable P. Standard deviations for three replicates are as follows: 2003 – H<sub>2</sub>O (1%), NaHCO<sub>3</sub> (5%), NaOH (2%), HCl (3%), and Residual (9%) and 2004 – H<sub>2</sub>O (3%), NaHCO<sub>3</sub> (3%), NaOH (5%), HCl (7%), and Residual (7%).

distribution of P fraction found in the manure used in 2003 was probably more typical of ruminant manure in general than that used in 2004 (Ajiboye et al., 2004; Barnett, 1994).

The manure amendments had a significant impact on soil properties (Table 2). Not only were

nutrient levels higher in amended soils, but also the pH values of amended soils were consistently 0.5 pH units higher than those in unamended soils. The higher pH in amended soils is likely a result of the high pH of the manure that was added to these soils. In addition, the manure is rich in nutrients, such as K, Ca, and P, leading to significantly higher concentrations of these nutrients in amended soils. As expected, soil organic matter concentration was also significantly higher in amended than in unamended soils.

Soil P<sub>tot</sub>, water-soluble P, P<sub>o</sub>, resin P, and the DPS are shown in Table 3. As expected, there was a significant increase in P<sub>tot</sub> in amended soils compared to unamended soils, due to the addition of P in the manure; however, P<sub>tot</sub> was not affected by rotation crop. Soil P<sub>o</sub> was not affected by amendment or crop (Table 3), suggesting that manure contributes primarily to the P<sub>i</sub> pool. Sharpley and Smith (1995) also concluded that soil P<sub>i</sub> fractions increased more than P<sub>o</sub> fractions following manure application. In addition, Sharpley et al. (2004) found P<sub>i</sub> fractions were significantly greater in soils receiving animal manure compared with untreated soils. Total water-soluble P levels ranged from 4.0 to 5.6 mg P kg<sup>-1</sup> (Table 3), and approximately 85% of the total water-soluble P was P<sub>i</sub> and presumably available for plant uptake (data not shown). These concentrations are higher than is necessary for optimal crop growth (Morgan, 1997). Water-soluble P was higher for forage plots than for potato or soybean plots (Table 3). There was a small but significant difference in DPS between amended (34%) and unamended (30%) soils, but no significant differences between crops. A DPS value of 25% is considered the critical level in the Netherlands (Sibbesen and Sharpley, 1997), indicating that P contamination of surface and groundwater is more likely to occur above this critical level. The high DPS in these soils, as well as the relatively high levels of water soluble P, indicate the potential for them to contribute P to nearby aquatic ecosystems.

Anion exchange resins remove P from soils while minimizing chemical alterations and pH changes (Olsen and Sommers, 1982); anion-exchange resin P is considered labile and part of the bioavailable P pool. There were significant differences in resin P due to both crop and amendment treatment (Table 3). Resin P was

Table 2. Soil test nutrient concentrations from amended and unamended plots in Maine potato ecosystem soils, in 2002 and 2003

Crop 2002	Crop 2003	Soil pH	P (mg kg <sup>-1</sup> soil)	K (mg kg <sup>-1</sup> soil)	Ca (mg kg <sup>-1</sup> soil)	OM <sup>a</sup> (%)
<i>Amended (+)</i>						
Barley	Forage	6.3a	33a	347ab	1560a	4.6a
Soybean	Barley	6.2a	28a	259b	1460a	4.3a
Potato	Soybean	6.2a	28a	308b	1110a	4.1a
Forage	Potato	6.3a	36a	466a	1610a	4.8a
<i>Unamended (-)</i>						
Barley	Forage	5.7a	18a	210ab	800a	2.4a
Soybean	Barley	5.9a	18a	233ab	920a	2.7a
Potato	Soybean	5.8a	16a	169b	890a	2.6a
Forage	Potato	5.9a	18a	270a	1010a	2.7a
Average(+)		6.2a	31a	345a	1510a	4.4a
Average(-)		5.8b	17b	220b	900b	2.6b
Crop 2003	Crop 2004	Soil pH	P (mg kg <sup>-1</sup> soil)	K (mg kg <sup>-1</sup> soil)	Ca (mg kg <sup>-1</sup> soil)	OM (%)
<i>Amended</i>						
Forage	Potato	6.4a	32a	430a	1750a	5.1a
Barley	Forage	6.4a	30a	374a	1560a	4.5ab
Soybean	Barley	6.2a	23a	286a	1310a	4.0b
Potato	Soybean	6.2a	25a	316a	1460a	4.4ab
<i>Unamended</i>						
Forage	Potato	5.8a	19a	335a	1030a	2.8a
Barley	Forage	6.0a	15a	199bc	890a	2.3a
Soybean	Barley	5.8a	15a	172c	820a	2.6a
Potato	Soybean	5.6a	15a	222b	780a	2.6a
Average(+)		6.3a	27a	352a	1520a	4.5a
Average(-)		5.8b	16b	232b	880b	2.5b

<sup>a</sup>OM = Organic Matter.

Means followed by the same letter are not significantly different ( $P = 0.05$ ). Mean separation done using Fisher's protected LSD test. K variable rank transformed in unamended soils in 2002 to meet the assumptions of ANOVA. Results reported on original scale. P and K variables log transformed in unamended soils in 2003 to meet the assumptions of ANOVA. Results reported on original scale.

doubled in amended compared to unamended soil, due to the addition of labile P with the manure. Forage had significantly higher concentrations of resin P than soybean or potato, and

barley had significantly higher concentrations than potato. Average resin P concentrations for the four rotation crops sampled in this study ranged from 14 to 20 mg P kg<sup>-1</sup>.

Table 3. Soil P fractions by rotation crop and soil treatment in Maine potato ecosystem plots

2003 Crop	WSP <sup>a</sup> (mg kg <sup>-1</sup> )	Resin P (mg kg <sup>-1</sup> )	DPS <sup>b</sup> (%)	Organic P (mg kg <sup>-1</sup> )	Total P (mg kg <sup>-1</sup> )
Potato	4.0b	14c	31a	207a	1730a
Soybean	4.0b	14bc	32a	184a	1740a
Barley	4.4ab	18ba	32a	264a	1830a
Forage	5.6a	20a	33a	223a	1830a
Unamended	3.2 b	11b	30b	207a	1670b
Amended	5.8a	22a	34a	232a	1890a

<sup>a</sup>WSP = water soluble phosphorus.

<sup>b</sup>DPS = degree of phosphorus saturation.

Means followed by the same letter are not significantly different, ( $P=0.05$ ) using Fisher's protected LSD.

No significant crop\*amendment interaction.

*Changes in soluble C and P during the growing season*

Amended soil had higher levels of soluble C than unamended soil at every sampling date (Table 4), indicating the role of manure additions in contributing to soluble C. When averaged over soil amendment, barley and forage plots had significantly higher concentrations of soluble C than soybean or potato in August 2003. In May 2004, soils from forage plots had significantly higher C concentrations than soils from barley, soybean, or potato plots. On July 2004 sampling date, the barley plots had the highest concentrations of soluble C, followed by forage and potato. The differences in soluble C concentrations between forage, barley and potato were not significant in May 2003 and August 2004 (Table 4). A significant crop\*amendment interaction was found in May 2004 (Table 4). However, it is difficult to determine the importance of this interaction, as it was the only significant interaction found in the five sampling dates.

Increased soluble C concentration associated with the barley and forage may be due to several factors. First, the forage plots undergo less soil disturbance from tillage and cultivation than the other crops. The barley, soybean, and potato plots are tilled prior to planting and the potato plots are cultivated during the growing season. The decrease in soil disturbance in the forage plots relative to other plots could promote soil C conservation (Reicosky et al., 1995). Amended potato plots receive more manure than other crops, yet the potato plots are consistently lower in soluble C than barley or forage plots suggesting that the high level of tillage and cultivation associated with potato production may be associated with decreased levels of soluble C in these plots. In addition, barley and forage are thought to be deep-rooted crops with relatively large root systems (Weaver, 1926). These large root systems may be contributing more soluble C to the soil directly through root exudates or indirectly through microbial action on insoluble C sources such as dead roots or sloughed root cells. Furthermore, the barley crop is under seeded with

Table 4. Soluble soil C and P concentrations by rotation crop and soil treatment in water extracts of soils from the Maine potato ecosystem plots in 2003 and 2004

	May 2003 (mg kg <sup>-1</sup> soil)	August 2003 <sup>a</sup> (mg kg <sup>-1</sup> soil)	May 2004 <sup>b</sup> (mg kg <sup>-1</sup> soil)	July 2004 <sup>c</sup> (mg kg <sup>-1</sup> soil)	August 2004 (mg kg <sup>-1</sup> soil)
<i>Soluble soil C</i>					
Potato	20.6a(6.2 <sup>d</sup> )	25.4c(5.8)	23.2b(6.6)	19.2c(6.1)	21.8a(5.5)
Soybean	21.2a(6.1)	28.6b(6.1)	23.4b(6.3)	—	—
Barley	20.0a(6.1)	33.4a(6.3)	22.6b(6.4)	28.4a(6.7)	24.0a(5.7)
Forage	22.0a(6.3)	33.0a(6.4)	26.4a(6.7)	25.4b(6.6)	26.0a(5.8)
Unamended	15.6b(6.1)	20.0b(6.0)	17.8b(6.3)	17.2b(6.2)	17.0b(5.4)
Amended	26.4a(6.3)	40.2a(6.4)	30.0a(6.7)	31.6a(6.7)	30.8a(5.9)
Interaction	ns	ns	<i>P</i> < 0.05	ns	ns
<i>Soluble soil P</i>					
Potato	1.0a	0.8b	0.6b	0.8b	1.0b
Soybean	1.4a	1.2a	0.6b	—	—
Barley	1.2a	1.4a	0.8b	1.2a	1.2ab
Forage	1.4a	1.4a	1.0a	1.2a	1.4a
Unamended	1.0b	0.6b	0.6b	0.8b	1.0b
Amended	1.6a	1.8a	1.0a	1.2a	1.4a
Interaction	ns	ns	ns	ns	<i>P</i> < 0.05

<sup>a</sup>Soluble C variable log transformed in August 2003 to meet the assumptions of ANOVA (data reported on original scale).

<sup>b</sup>P variable log transformed in May 2004 to meet the assumptions of ANOVA (data reported on original scale).

<sup>c</sup>P variable rank transformed in July 2004 to meet the assumptions of ANOVA (data reported on original scale).

<sup>d</sup>Soil pH values in parenthesis.

Means followed by the same letter are not significantly different (*P* = 0.05) using Fisher's protected LSD test.



Table 5. Correlation coefficients ( $r$ ) in amended and unamended soils in Maine potato ecosystem plots in 2003 and 2004

	May-03	Aug-03	May-04	Jul-04	Aug-04
<i>Soluble C vs. pH</i>					
Unamended	0.09ns	0.77*	-0.08ns	0.80*	0.50ns
Amended	0.36ns	0.46ns	0.48ns	0.78*	0.59*
All plots	0.59*	0.77*	0.79*	0.85*	0.85*
<i>Soluble C vs. Soluble P</i>					
Unamended	-0.20ns	0.80*	0.19ns	-0.13ns	0.02ns
Amended	0.14ns	0.58*	0.26ns	0.62*	0.35ns
All plots	0.48*	0.87*	0.65*	0.53*	0.60*
<i>Soluble P vs. pH</i>					
Unamended	0.41ns	0.51*	0.09ns	0.26ns	0.02ns
Amended	0.65*	0.73*	0.50*	0.80*	0.77*
All plots	0.69*	0.78*	0.68*	0.59*	0.67*

ns, Not significant ( $P=0.05$ ).

\*Significant ( $P=0.05$ ).

$n=32$  in May and August 2003 and May 2004 in all plots ( $n=16$  in amended and unamended plots).

$n=24$  in July and August 2004 in all plots ( $n=12$  in amended and unamended plots).

an alfalfa/timothy mix, which is contributing additional C to the soil through increased biomass. Although the effect of manure additions on levels of soluble C is large, there are also differences in soluble C concentrations between crops in both amended and unamended soils, suggesting that plant root systems also influence levels of soluble soil C.

Amended soils had higher levels of soluble P than unamended soils at every sampling date (Table 4). In May 2003, there were no significant differences in P levels between crops; however, there were significant differences at all other sampling dates. A significant crop\*amendment interaction was found in August 2004 (Table 4). However, as with soluble C, the importance of this interaction is questionable as no other interaction was found to be significant. Averaged over amendment, forage plots had the highest levels of soluble P at all sampling dates (shared the highest level with soybean in May 2003 and with barley in August 2003 and July 2004), while potato plots had the lowest levels of soluble P at all sampling dates (Table 4). In addition to the potato plots receiving the most manure (amended plots), the unamended potato plots also received the highest rate of inorganic P fertilizer. Thus, it could be expected that the potato plots would have the highest soluble P concentrations. Levels of soluble soil P could also be affected by plant uptake of P, a factor that was not mea-

sured in our study. However, the fact that soluble P concentrations were consistently higher in barley and forage than in potato plots supports the hypothesis that the higher levels of soluble C from barley and forage plots are increasing P solubility relative to potato plots.

The addition of manure increased soil pH, soluble P, and soluble C relative to unamended soil; therefore correlations are presented for amended and unamended soils separately, as well as all soils together (Table 5). For all soils together correlations between soluble C and soluble P were significant at all sampling dates. Considering amended and unamended soils separately, correlation coefficients were typically higher in amended than in unamended soils, although not all of these correlations were significant. Significant correlations between soluble C and P in amended soils were found for August 2003 ( $r=0.58$ ) and July 2004 ( $r=0.62$ ), and in unamended soils for August 2003 ( $r=0.80$ ). All other correlations between C and P were not significant, with  $r$  values ranging from 0.69 in May 2004 to 0.89 in August 2004 (Table 5). The generally higher correlation coefficients in amended soils suggest that the manure is improving the relationship between C and P. One proposed mechanism for this process is through ligand-exchange reactions, whereby low molecular weight organic acids desorb P from soil surfaces (Fox et al., 1990).

The solubility of P is influenced by soil pH, with the maximum P solubility in soils generally occurring between pH 6.0 and 7.0 (Lindsay and Moreno, 1960). Soil pH and soluble P concentration were positively correlated at all five sampling dates when amended and unamended soils were considered together, and for amended soils considered alone. pH values ranged from 5.4 to 6.7 with unamended soils lower than amended soils (Table 4). In separate laboratory experiments we added HCl to amended soils to lower pH (0.55 and 0.33 units) and raised pH of unamended soils with NaOH (0.29 and 0.45 units). There were no significant changes in soil P concentrations due to pH manipulation (data not shown) suggesting that pH itself is not the determining factor affecting soil P concentration over the limited pH range of these samples.

Soluble C was also strongly correlated with pH at all sampling dates, considering unamended and amended soils together (Table 5). Again, the manure increases both pH and soluble C levels, and may improve the relationship between these two variables in amended soils. However, even in unamended soils soluble C levels are expected to increase to some degree with an increase in pH. Low soil pH promotes several reactions that are thought to be involved in sorption of soluble soil organic matter by soil surfaces including ligand exchange, protonation, and cation bridging (Sposito, 1989).

#### *Root length estimates and relationship with soluble C*

Average RLD obtained using the line-intercept method in 2003 ranged from 1.2 to 2.7  $\text{cm cm}^{-3}$  (Figure 2a). In 2004 average values obtained using the WinRHIZO scanner ranged from 3.1 to 10.1  $\text{cm cm}^{-3}$  (Figure 2b,c). We measured four samples from 2004 by both methods in order to compare the two methods directly on the same samples. For potato RLD values ( $\text{cm cm}^{-3}$ ) were 4.8 (WinRHIZO, WR) versus 1.0 (line-intercept, LI), for forage 10.2 (WR) versus 2.3 (LI), and for barley 6.3 and 7.4 (WR) versus 2.4 and 2.8 (LI), clearly suggesting that the WinRHIZO method yields higher values than the line-intercept method. A range of RLD values are common. For example, Heeraman and Juma (1993), Lampurlanes et al. (2002), Opena and Porter

(1999), and Parker et al. (1989) all report RLD between 1 and 5  $\text{cm cm}^{-3}$  for barley and potato, measured using the line-intercept method. Using the WinRHIZO scanner, Vaughan et al. (2002) measured 6–12  $\text{cm cm}^{-3}$  for alfalfa RLD.

Despite the differences in magnitude of root length between 2003 and 2004, which was likely the result of the two different measurement methods as well as perhaps different growing condi-

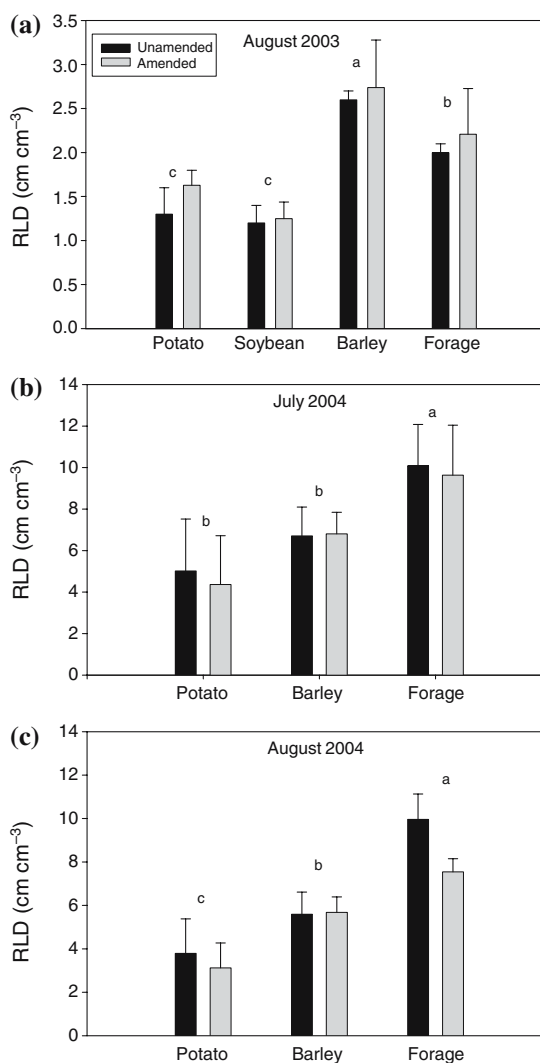


Figure 2. Crop root length density (RLD) in amended and unamended soils of the Maine potato ecosystem project measured in August 2003 and July and August 2004. No significant crop\*amendment interaction found at any sampling date. RLD significantly higher in unamended crops than amended crops in August 2004, no significant difference in August 2003 or July 2004.

tions between years, the basic trends between crops were similar in both years; barley and forage had significantly higher RLD than potato or soybean. In 2003, barley had the highest RLD, followed by forage, potato and soybean (Figure 2a). In 2004, forage had the highest RLD in both July and August, followed by barley and potato (Figure 2b, c). Potato had the lowest RLD in July and August 2004, and potato was not significantly different than soybean in 2003. The differences in root morphology and architecture between these crops may partly explain the differences found in root lengths. Dicots, such as alfalfa, potato, and soybean, have a central taproot from which lateral roots emerge (Tinker and Nye, 2000). However, the potatoes used in this study were propagated vegetatively, therefore they have only adventitious roots and no taproot. The taproot (and adventitious roots in the case of the potatoes) thickens as the plant matures due to cambial activity. Conversely, monocots, like barley, have a fibrous root system where seminal roots develop from the germinating seed and nodal roots arise from the shoot (Tinker and Nye, 2000). Typically, fibrous root systems will have more fine roots and will extend deeper into the soil than taproot systems. However, alfalfa is known for its ability to extend its taproot deep into the soil (Russell, 1977).

Although root morphology dictates RLD to a certain extent, soil and environmental conditions also play an important role in root development. Soil water, soil temperature, bulk density, oxygen supply, and nutrient supply can all influence root growth (Tinker and Nye, 2000). Bulk density and soil moisture were measured in all soils collected in this study and only minimal differences were found between crops. Both soil bulk density ( $0.92\text{--}1.18\text{ g cm}^{-3}$ ) and soil water content (20.5–28.2%) were typical for a cultivated loamy soil (Brady and Weil, 1999) and should not have significantly affected root growth. Despite soil amendment effects on soil properties, RLD was not significantly affected by soil amendment (Figure 2). In contrast, Opena and Porter (1999) found that compost and manure amendment increased potato RLD in a similar study conducted in 1993 and 1994. The difference between Opena and Porter's results and those reported here may be due to different potato varieties, different nutrient levels in the plots and, perhaps most

importantly, to the fact that 1993 (127 mm precipitation in July and August) and 1994 (113 mm) were significantly drier years than 2003 (203 mm) and 2004 (174 mm). Amendments are likely to improve both root growth and yield more in drier years than wetter years due to increases in soil organic matter content and soil moisture with amendment (Lotter et al., 2003).

There was a noticeable decline in RLD between July and August, 2004. For all crops, RLD decreased at least  $1\text{ cm cm}^{-3}$  between July and August. The largest decline was seen in amended forage plots, with a  $2.1\text{ cm cm}^{-3}$  decrease in RLD. In part, this decline can be attributed to root death and turnover. In annual crops, root biomass typically decreases far in advance of harvest (Tinker and Nye, 2000). Such environmental factors as soil temperature, soil moisture, solar radiation, and soil nutrient levels can also influence root turnover (Lauenroth and Gill, 2003), and may have played a role in the decrease in RLD between July and August.

Despite the fact that the crops with higher root lengths typically had the highest levels of soluble C, the correlation between RLD and soluble C was significant at only one sample date (August 2003) for amended plots (Figure 3). Measurements of RLD are quite variable with CVs of 50–100% not uncommon (Do Rosario et al., 2000), and high measurement variability may have contributed to low  $r$  values in this study. Although roots may influence soluble soil C levels, they are not the sole contributor to soil C levels. As mentioned previously, the crops with the longest roots (barley and forage) also undergo the least amount of soil disturbance from cultural practices, which may lessen the amount of C lost to the atmosphere as  $\text{CO}_2$ . In addition, the forage crop overwinters, which may increase soil C levels. Overall root contributions to the soil C pool may be substantial and important, but the measurable level of soluble C may decrease dramatically beyond the rhizosphere. Root exudates are subject to physical, chemical and biological changes within the rhizosphere, including sorption, oxidation, and microbial degradation (Inderjit and Weston, 2003). Jones et al. (2003) postulated that soil microbes are actively scavenging soluble soil C, thereby limiting accumulation in soil. Cheng et al. (1994) used  $^{14}\text{C}$  pulse labeling to determine that only 2–3% of root

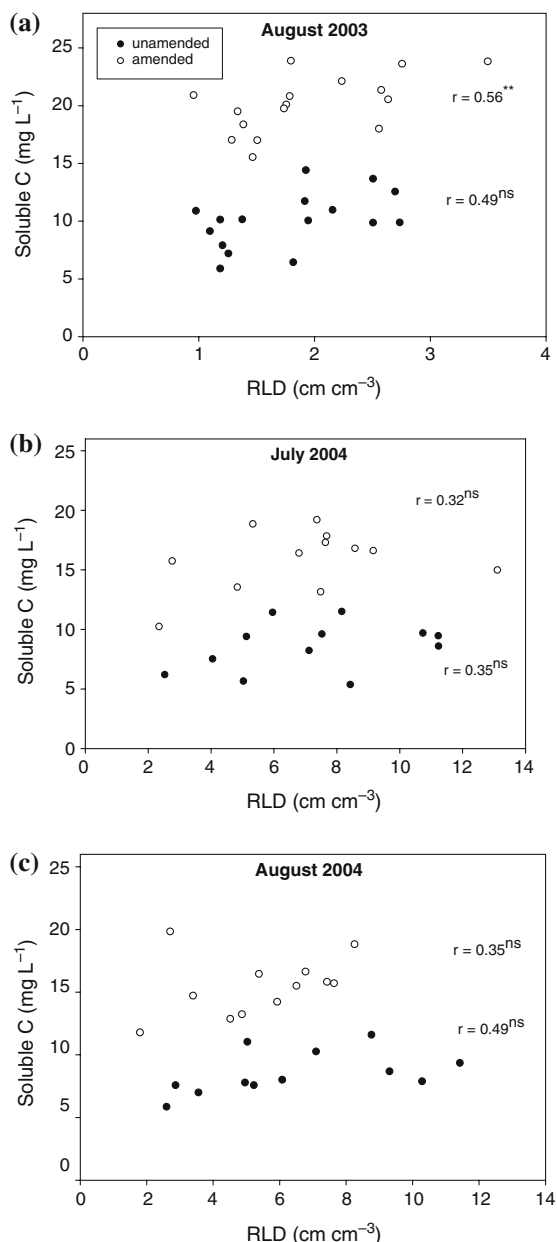


Figure 3. Correlation between soluble soil C concentrations and crop root length density (RLD) in amended and unamended soils of the Maine potato ecosystem project in August 2003 and July and August 2004.

exudates were found in the bulk soil. Similarly, Gregory and Atwell (1991) found that only 4.1% of the total C input to the soil was recovered as water-soluble exudates for barley. Thus, soluble C levels in rhizosphere soil may show a stronger relationship to RLD than soluble C levels in the bulk soil.

## Conclusion

There were differences among crops in root growth. Barley and forage consistently had higher RLD than potato or soybean crops. Barley and forage plots also typically had higher concentrations of soluble soil C during the growing season than potato or soybean, but the differences were significant at only three of the five sampling dates. RLD was significantly correlated to soluble C only for amended soils on the August 2003 sampling date. As with soil C, soluble soil P levels were typically higher in barley and forage plots than in soybean and potato. When dried soil samples were extracted, forage had the highest levels, and potato and soybean had the lowest levels, of both water-soluble P and resin-extractable P, suggesting that P was more bioavailable in forage plots. It is possible that soluble C derived from root systems in the forage plots may have sorbed to soil surfaces altering them in a way that made P more soluble and extractable. Despite the difficulty of measuring root length in soils and the numerous dynamic processes which may influence both soluble C and soluble P, our study shows the importance of root influences on soil chemical processes and suggests the possibility of soil soluble C fraction involvement in P chemistry.

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